

THAT WHICH IS CLAIMED:

1. An endotoxin comprising at least one engineered cathepsin-sensitive proteolytic site, wherein said endotoxin has improved pesticidal activity relative to an  
5 endotoxin which lacks said at least one cathepsin-sensitive proteolytic site.

2. The endotoxin of claim 1, further comprising at least one mutation consisting of an alteration of at least one other proteolytic site whereby the stability in an insect gut of said endotoxin containing said at least one mutation is increased relative to the stability  
10 of an endotoxin lacking said at least one mutation.

3. The endotoxin of claim 2, wherein alteration of at least one other proteolytic site consists of replacing at least one amino acid of said at least one other proteolytic site with valine.  
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4. The endotoxin of claim 1, wherein said at least one engineered cathepsin-sensitive proteolytic site comprises the amino acid sequence FRSRG.

5. The endotoxin of claim 1, wherein said endotoxin comprises at least two  
20 engineered cathepsin-sensitive proteolytic sites.

6. The endotoxin of claim 1, wherein said endotoxin further comprises an alteration in which an additional amino acid adjacent to at least one of said at least one engineered cathepsin-sensitive proteolytic site is mutated from a wild type sequence so  
25 that proteolysis at said at least one engineered cathepsin-sensitive proteolytic site is enhanced in comparison to an endotoxin which does not contain said alteration.

7. The endotoxin of claim 1, wherein said at least one engineered cathepsin-sensitive proteolytic site is FRSRG and said endotoxin comprises an amino acid sequence  
30 FRSRGFRSRGP.

8. The endotoxin of claim 1, wherein said at least one engineered cathepsin-sensitive proteolytic site comprises an amino acid sequence FRRG.

5 9. The endotoxin of claim 5, wherein said endotoxin comprises an amino acid sequence FRRGFRRG.

10. A method of enhancing pesticidal activity of an endotoxin comprising adding at least one engineered cathepsin-sensitive proteolytic site to said endotoxin.

10 11. The method of claim 10, wherein said at least one engineered cathepsin-sensitive proteolytic site comprises an amino acid sequence FRSRG.

12. The method of claim 10, further comprising adding at least one other proteolytic site to said polypeptide.

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13. The method of claim 10, wherein said at least one engineered cathepsin-sensitive proteolytic site comprises an amino acid sequence FRRG.

20 14. A method of increasing the pesticidal activity of a pesticidal polypeptide comprising altering at least one proteolytic site in said pesticidal polypeptide by replacing at least one amino acid of said at least one proteolytic site with a different amino acid, whereby the stability of said pesticidal polypeptide in an insect gut is increased relative to a pesticidal polypeptide which lacks said at least one altered proteolytic site.

25 15. The method of claim 14, further comprising adding at least one engineered proteolytic site to said pesticidal polypeptide, wherein said pesticidal polypeptide has improved pesticidal activity relative to a pesticidal polypeptide which lacks said at least one additional engineered proteolytic site.

16. The method of claim 14, wherein said at least one altered proteolytic site is selected from the group consisting of a trypsin site, a chymotrypsin site, and a cathepsin-like site.

5           17. The method of claim 15, wherein said additional engineered proteolytic site is selected from the group consisting of a trypsin site, a chymotrypsin site, and a cathepsin-like site.

10           18. The method of claim 15, wherein said additional at least one engineered proteolytic site is a chymotrypsin site and said altered at least one proteolytic site is a chymotrypsin site.

19. The method of claim 14, wherein said pesticidal polypeptide is pentin-1.

15           20. An endotoxin comprising a mutation consisting of the alteration of at least one proteolytic site, whereby the stability of the endotoxin in an insect gut is increased relative to an endotoxin lacking said mutation.

20           21. The endotoxin of claim 20, wherein said mutation consists of an alteration selected from the group consisting of:

- a) an addition of at least one amino acid to at least one proteolytic site;
- b) a removal of at least one amino acid from at least one proteolytic site;
- c) a replacement of at least one amino acid of at least one proteolytic site with a different amino acid; and
- 25           d) a combination of at least two of (a), (b), and (c).

22. The endotoxin of claim 21, wherein said mutation consists of an alteration which is the replacement of at least one amino acid of a proteolytic site with an amino acid selected from the group consisting of valine and arginine.

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23. An endotoxin comprising a mutation wherein a sequence within said endotoxin is altered by replacing at least one amino acid with a valine, wherein said sequence comprises ITTLNLATDSSLALKHNLGED.

5           24. The endotoxin of claim 23, wherein said sequence is altered to the second sequence ITTLNLATDSSLALKHNVGED.

25. The endotoxin of claim 23, wherein said sequence is altered to the second sequence ITTLNLATDSSLAVKHNVGED.

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26. The endotoxin of claim 23, wherein said sequence is altered to the second sequence ITTVNLATDSSVAVKHNLGED.

27. The endotoxin of claim 23, wherein said sequence is altered to the second  
15 sequence ITTVNLATDSSVAVKHNVGED.

28. An endotoxin comprising a mutation wherein a first sequence DYKDYLKMSAGN within said endotoxin is altered by replacing said first sequence with the second sequence DYKDYAVGSAGN.

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29. An endotoxin comprising a mutation wherein a first sequence INNYDRQ within said endotoxin is altered by replacing said first sequence with the second sequence INNVDRQ.

25           30. An endotoxin comprising a mutation wherein a first sequence NYDTRTYPMETKA within said endotoxin is altered by replacing said first sequence with the second sequence NYDTITYPIETKA.

31. An isolated nucleic acid comprising a nucleotide sequence selected from the  
30 group consisting of:

(a) a nucleotide sequence set forth in SEQ ID NO:21, 25, 29, 33, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, or 93;

5 (b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;

(c) a nucleotide sequence encoding an amino acid sequence corresponding to domain 1 of the protein encoded by SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 10 84, 86, 88, 90, 92, or 94;

(d) a nucleotide sequence encoding an amino acid sequence corresponding to domain 2 of the protein encoded by SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;

15 (e) a nucleotide sequence characterized by at least 88% sequence identity to a nucleotide sequence of (a);

(f) a nucleotide sequence encoding a protein comprising an amino acid sequence characterized by at least 85% sequence identity to an amino acid sequence of (b);

20 (g) an antisense nucleotide sequence complementary to a nucleotide sequence of any one of (a) to (d); and

(h) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of (a).

25 32. The nucleic acid according to claim 31, wherein said nucleotide sequence is optimized for expression in a plant.

33. An expression cassette comprising a nucleic acid according to claim 31, wherein said nucleotide sequence is operably linked to a promoter that drives expression 30 in a microorganism or in a plant cell.

34. An isolated pesticidal polypeptide selected from group consisting of:

(a) a polypeptide comprising an amino acid sequence set forth in  
SEQ ID NO:22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66,  
68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94; and

5 (b) a polypeptide characterized by at least 85% sequence identity to an  
amino acid sequence of (a).

35. The polypeptide according to claim 34, wherein said polypeptide is  
characterized by pesticidal activity against at least one pest belonging to the order  
10 Coleoptera.

36. A pesticidal composition comprising at least one polypeptide according to  
claim 34 in combination with a carrier.

15 37. The pesticidal composition of claim 36, further comprising an additional  
*Bacillus thuringiensis* toxin.

38. The pesticidal composition of claim 37, wherein said additional *Bacillus*  
*thuringiensis* toxin is a *Cry3B* toxin.

20 39. A method for impacting an insect pest comprising applying the pesticidal  
composition according to claim 36 to the environment of the insect pest by a procedure  
selected from the group consisting of spraying, dusting, broadcasting, and seed coating.

25 40. The method according to claim 39, wherein said insect pest is selected from  
the group consisting of Colorado potato beetle, western corn rootworm, southern corn  
rootworm, and boll weevil.

30 41. A transformed plant comprising in its genome at least one stably incorporated  
nucleotide construct comprising a coding sequence operably linked to a promoter that

drives expression of a polypeptide that is pesticidal for at least one pest belonging to the order Coleoptera, wherein said coding sequence is selected from the group consisting of:

- 5 (a) a nucleotide sequence set forth in SEQ ID NO:21, 25, 29, 33, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, or 93;
- (b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;
- 10 (c) a nucleotide sequence encoding an amino acid sequence corresponding to domain 1 of the protein encoded by SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;
- (d) a nucleotide sequence encoding an amino acid sequence corresponding to domain 2 of the protein encoded by SEQ ID NO: 22, 26, 30, 34, 15 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;
- (e) a nucleotide sequence characterized by at least 88% sequence identity to a nucleotide sequence of (a);
- (f) a nucleotide sequence encoding a protein comprising an amino 20 acid sequence characterized by at least 85% sequence identity to an amino acid sequence of (b);
- (g) a nucleotide sequence according to any one of (a) to (d) that comprises codons optimized for expression in a plant;
- (h) an antisense nucleotide sequence complementary to a nucleotide 25 sequence of any one of (a) to (d); and
- (i) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of (a).

42. The plant of claim 41, wherein said plant further comprises in its genome a  
30 second stably incorporated nucleotide construct comprising a second coding sequence that encodes a polypeptide that is pesticidal for at least one pest.

43. The plant of claim 42, wherein said second coding sequence encodes a polypeptide selected from the group consisting of: *Cry3A* and *Cry3B*.

5           44. The plant of claim 41, wherein said plant is a monocot.

45. The plant of claim 41, wherein said plant is a dicot.

46. Transformed seed of the plant of claim 41.

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47. A transformed microorganism comprising a nucleotide sequence selected from the group consisting of:

          (a)     a nucleotide sequence set forth in SEQ ID NO:21, 25, 29, 33, 39,  
41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83,  
15           85, 87, 89, 91, or 93;

          (b)     a nucleotide sequence encoding the amino acid sequence set forth  
in SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64,  
66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;

          (c)     a nucleotide sequence encoding an amino acid sequence  
20           corresponding to domain 1 of the protein encoded by SEQ ID NO: 22, 26, 30, 34,  
40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82,  
84, 86, 88, 90, 92, or 94;

          (d)     a nucleotide sequence encoding an amino acid sequence  
corresponding to domain 2 of the protein encoded by SEQ ID NO: 22, 26, 30, 34,  
25           40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82,  
84, 86, 88, 90, 92, or 94;

          (e)     a nucleotide sequence characterized by at least 88% sequence  
identity to a nucleotide sequence of (a);

          (f)     a nucleotide sequence encoding a protein comprising an amino  
30           acid sequence characterized by at least 85% sequence identity to an amino acid  
sequence of (b);



(g) an antisense nucleotide sequence complementary to a nucleotide sequence of any one of (a) to (d); and

(h) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of (a).

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48. The transformed microorganism of claim 47, wherein said nucleotide sequence is operably linked to a promoter that drives expression in said microorganism.

49. A pesticidal composition comprising a transformed microorganism according to claim 47 and a carrier.

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50. A method of impacting a pest comprising applying the pesticidal composition according to claim 49 to the environment of the pest by a procedure selected from the group consisting of spraying, dusting, broadcasting, and seed coating.

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51. A method for impacting a pest of a plant comprising introducing into said plant or cell thereof at least one nucleotide construct comprising a sequence that encodes a pesticidal polypeptide operably linked to a promoter that drives expression of a polypeptide in plant cells, wherein said nucleotide sequence is selected from the group consisting of:

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(a) a nucleotide sequence set forth in SEQ ID NO:21, 25, 29, 33, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, or 93;

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(b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;

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(c) a nucleotide sequence encoding an amino acid sequence corresponding to domain 1 of the protein encoded by SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;

- (d) a nucleotide sequence encoding an amino acid sequence corresponding to domain 2 of the protein encoded by SEQ ID NO: 22, 26, 30, 34, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, or 94;
- 5 (e) a nucleotide sequence characterized by at least 88% sequence identity to the nucleotide sequence set forth in (a);
- (f) a nucleotide sequence encoding a protein comprising an amino acid sequence characterized by at least 85% sequence identity to an amino acid sequence of (b);
- 10 (g) an antisense nucleotide sequence complementary to a nucleotide sequence of any one of (a) to (d); and
- (h) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of (a).

15 52. The method of claim 51, wherein the plant produces a pesticidal polypeptide characterized by pesticidal activity against at least one pest of the order Coleoptera.

53. The method according to claim 52, wherein said plant pest is selected from the group consisting of Colorado potato beetle, western corn rootworm, southern corn  
20 rootworm, and boll weevil.

54. A method of predicting potential areas for modification in the amino acid sequence of a naturally occurring pesticidal protein to enhance pesticidal activity comprising:

25 subjecting said naturally occurring pesticidal protein to proteolytic digestion to provide a digested pesticidal protein;

assaying said digested pesticidal protein for a change in pesticidal activity relative to the pesticidal activity of the naturally occurring pesticidal protein;

determining one or more proteolytic sites for modification;

30 modifying the amino acid sequence of said naturally occurring pesticidal protein at said one or more proteolytic sites wherein said pesticidal activity is enhanced.

55. The method of claim 54 wherein said proteolytic digestion occurs *in vivo*.

56. The method of claim 54 wherein said proteolytic digestion occurs *in vitro*.

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